Freshwater snails (Mollusca: Gastropoda) from the Commonwealth of Dominica with a discussion of their roles in the transmission of parasites

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Abstract: We collected six species of freshwater snails from Dominica, including Biomphalaria kulmiana (Clessin, 1883), Gundlachia radiata (Guilding, 1828), Helisoma (=Planorbella) trivolvis (Say, 1817), Melanoides tuberculata (Müller, 1774), Neritina punctulata Lamarck, 1816, and Physa marmorata Guilding, 1828. Our collections indicate that un-reported species such as G. radiata and H. trivolvis are established on Dominica, West Indies. We tested a limited number of M. tuberculata for rickettsial pathogens, Neorickettsia spp., but did not identify this agent. Three species of snails previously reported from Dominica, Biomphalaria glabrata (Say, 1818), Biomphalaria straminea (Dunker, 1848), and Thiara granifera (Lamarck, 1822), were not collected. Our data suggest that B. glabrata has not re-emerged as a prominent component of the freshwater snail fauna since it disappeared or was locally eradicated. In addition, previous reports of B. straminea were probably misidentifications of B. kulmiana, and some abnormally large specimens of M. tuberculata from Freshwater Lake could be misidentified as T. granifera. Our sampling was not adequate to demonstrate that T. granifera was absent from Dominica. We determined that B. kulmiana was not eradicated by previous molluscan control regimes. Additional studies on the relationships of freshwater snails in Dominica to helminths of animals and humans are needed to understand the public and veterinary health significance of these snails.

Key words: Biomphalaria, Gundlachia, Helisoma, Physa, West Indies

The Commonwealth of Dominica is a small (790 km²) mountainous island nation in the West Indies that receives over 900 cm of rain per year (Grell 1976). The freshwater snail fauna of Dominica has been studied in regard to its significance in the transmission of schistosomiasis (e.g., Noblet and Damian 1991), but the snail fauna has been largely ignored in other regards. Freshwater snails are the primary intermediate hosts for most trematodes, some nematodes, and some rickettsial pathogens (Neorickettsia spp.). There have been no reports of autochthonous schistosomiasis on Dominica, but visitors and immigrants harboring the worm have been documented (Grell 1976, Prentice 1980, Grell et al. 1981, Noblet and Damian 1991, Adedayo and Nasiiro 2004). There remains a potential for transmission and establishment of schistosomiasis as long as susceptible populations of Biomphalaria Preston, 1910 are established on the island. Biomphalaria glabrata (Say, 1818) was reported on Dominica (Prentice 1980) but more recent surveys (Noblet and Damian 1991) indicate that this snail was replaced by Biomphalaria straminea (Dunker, 1848). In addition, two molluscan intermediate hosts of the trematode Paragonimus westermani were introduced on Dominica (Noblet and Damian 1991). The potential to establish this trematode is relatively small because it is not established on neighboring islands. Prosobranch molluscs are the intermediate hosts for trematodes that transmit Neorickettsia spp. to humans and

domestic animals throughout the Americas (Pusterla et al. 2000, Headley et al. 2004). Neorickettsia spp. have not been reported from Dominica. We conducted a survey of freshwater ponds, lakes, and rivers to determine the distribution of freshwater snails on Dominica. We tested selected Melanoides tuberculata (Müller, 1774) for the presence of Neorickettsia by PCR amplification of the 16S rRNA gene of Neorickettsia.

MATERIALS AND METHODS

Snails were collected (sites listed in Table 1) by removing them from vegetation and mud using nets and sieves or by snorkeling in streams and removing them from rocks. All specimens were preserved in 99% ethanol, which was changed completely after 24 hours.

All snails were identified with morphological characters. DNA was extracted from individual specimens in the genus *Biomphalaria* and 9 *Melanoides tuberculata* from each collection locality. The DNA extraction, PCR, PCR cleanup, and sequencing followed the techniques described by Reeves *et al.* (2006) with the following modifications. We extracted DNA from individual snails and amplified the internal transcribed spacer 2 (ITS-2) and a portion of the 28S rRNA gene from each specimen of *Biomphalaria*, using the primers de-

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Table 1. Collection data and snail species identified from sites in Dominica, West Indies in 2005.

Collection site	Habitat type	Collection date	Species collected
Springfield Estate, Parish of Sainte Paul	Pond and outflow stream	12-17 May 2005	Biomphalaria kulıniana Melanoides tuberculata Neritinia punctulata
Roseau Botanic Garden, Roseau, Parish of Sainte George	Artificial pond	13 May 2005	Physa marmorata B. kulıniana Helisoına trivolvis M. tuberculata
Dancey and cover drains	Standing water	12 May 2005	P. marmorata B. kuhniana
Roseau, open sewer drains, Parish of Sainte George	Standing water	13 May 2005	P. marmorata
Roseau River, Roseau, Parish of Sainte George	River	13 May 2005	N. punctulata
Clark Hall River, Parish of Saint Paul	River	14 May 2005	Gundlachia radiata M. tuberculata N. punctulata
Freshwater Lake, Parish of Saint George	Lake	15 May 2005	B. kulmiana M. tuberculata
Miranda's Pond, Parish of Saint George	Pond	16 May 2005	P. marmorata B. kuhniana N. punctulata
Cochran, unnamed pond, Parish of Sainte Paul	Pond	16 May 2005	G. radiata
Middleham Falls, Parish of Sainte Paul	River	16 May 2005	N. punctulata

scribed by Caldeira *et al.* (2004). DNA extracts from *M. tuberculata* were screened for DNA of *Neorickettsia* by PCR using the EHR16SD and EHR16SR PCR primers to amplify the 16S rRNA gene of *Neorickettsia* as described by Inokuma *et al.* (2000). Positive controls with DNA from *Helisoma trivolvis* (Say, 1817) or *Wolbachia* sp. and a negative control with distilled water were used. We used positive controls that could be amplified by PCR but represented organisms other than those examined in our study.

Voucher specimens of snails were deposited in the Academy of Natural Sciences of Philadelphia. DNA sequences for the ITS-2 and a portion of the 28S rRNA gene of *Biomphalaria kuhniana* (Clessin, 1883) (GenBank Accession #DQ111952) were deposited in GenBank.

RESULTS AND DISCUSSION

We did not collect *Biomphalaria glabrata* or *Biomphalaria straminea* but did collect *Biomphalaria kuhniana* from isolated ponds and Freshwater Lake (Table 1). The DNA sequences for the ITS-2 and a portion of the 28S rRNA gene from our specimens were 100% identical to those of *B. kuhniana* (GenBank #s AY030380, AY030378, AY030379) from Columbia, Dominica, and Venezuela. *Biomphalaria kuhni-*

ana was described from a "Chinese well" in Panama and is morphologically similar to B. straminea (Paraense 2003). The two species can be separated with molecular and morphological characters. Biomphalaria kuhniana is not a competent intermediate host for Schistosoma mansoni (Paraense 2003), which could explain why schistosomiasis has not become established on Dominica even though Biomphalaria and occasional transient human infections with S. mansoni have been reported in Dominica. Noblet and Damian (1991) reported populations of B. straminea in artificial ponds on the island but not from Freshwater Lake. However, we suggest that the previous reports of B. straminea were misidentifications of B. kuhniana, which was not included in the key by Malek (1985), used to diagnose snails in the previous surveys. In addition we collected B. kuhniana in Freshwater Lake, which is a new locality for this snail. DeJong et al. (2001) had reported a population of B. kuhniana from Roseau, Dominica. Biomphalaria kuhniana is not known to serve as the intermediate host of trematodes parasitic to humans or domestic animals; however, little research has focused on the possibility that this snail is a host to helminths other than S. mansoni. Other species of Biomphalaria are intermediate hosts to echinostomatid trematodes and nematodes in the genus Angiostrongylus (Malek 1980).

We collected ancylid limpets in both streams and ponds.

All limpets were morphologically identified as *Gundlachia* radiata (Guilding, 1828), which has not been previously reported from Dominica, but is known from neighboring islands (Starmuhler 1984, Malek 1986). *Gundlachia radiata* is not considered an intermediate host to trematodes of medical significance. It does harbor anisakid nematodes, which are parasitic to fish and some fish-eating mammals (Thiengo *et al.* 2000). Ancylidae are small and often go unnoticed by collectors. These limpets might play important roles in the natural cycles of helminths in Dominica but are currently unstudied.

Helisoma trivolvis (Say, 1817), a planorbid snail that could be mistaken for Biomphalaria by untrained collectors, was collected in the metal ponds at the Roseau Botanic Garden. Helisoma trivolvis is established in the Dominican Republic and possibly Haiti and Cuba (Ayvazian and Mallett 1986, Paraense 2003). A congeneric species, Helisoma duryi (Wetherby, 1879) is also established in the Caribbean (e.g., Pointier 2001). Helisoma trivolvis is naturally resistant to infection by Schistosoma mansoni (Ayvazian and Mallett 1986), but this snail is an intermediate host to clinostomatid, cyclocoeliid, echinostomatid, and strigeid trematodes and is used as a laboratory host to nematodes in the genus Angiostrongylus (Malek 1980, Ponder and Fried 2004). Humans and domestic animals can be infected by some of these worms, including Alaria canis, which can cause fatal infections in humans (Malek 1980). As with other zoonotic trematodes, infections of humans are accidental and usually involve eating uncooked meat harboring metacercariae.

We did not collect Thiara granifera (Lamarck, 1822), but the specimens of Melanoides tuberculata from Freshwater Lake were abnormally large and were initially misidentified as T. granifera, Melanoides tuberculata was collected in both streams and ponds. We did not amplify DNA from Neorickettsia spp. in any of our collections of M. tuberculata. This exotic snail was possibly introduced to Dominica around 1975 (Pointier and McCullough 1989). Melanoides tuberculata is a potential biological control agent for Bioniphalaria spp. because the two snails appear to compete, and M. tuberculata might exclude Biomphalaria spp. in some habitats (Pointier and McCullough 1989). Our data indicate that exclusion does not occur in Dominica (Table 1). Melanoides tuberculata is not a suitable host for Schistosoma mansoni but is an intermediate host to Paragoninus westermani, a lung fluke. There is a possibility that P. westermani or other Paragonimus spp. will become established on Dominica, because both M. tuberculata and the freshwater-crab, secondintermediate hosts of P. westermani, are present (Noblet and Damian 1991). Carnivorous mammals are natural hosts for this fluke so zoonotic cycles of the parasite could become established without human infections. *Melanoides tuberculata* can serve as the intermediate host to other trematodes that occasionally infect humans, including eye flukes (*Philophthalnus* spp.) of birds (Dimitrov *et al.* 2000, Lamothe-Argumendo *et al.* 2003). *Melanoides tuberculata* is also an intermediate host to *Heterophyes heterophyes*, a fluke of fish-eating mammals and birds (Malek 1980). *Heterophyes heterophyes* can infect humans.

Physa marmorata Guilding, 1828 was collected from ponds and water tanks with freshwater plants. Physid snails are often overlooked as intermediate hosts of helminths, but P. marmorata is the intermediate host for the echinostomatid trematodes, Echinostoma huisreyi and Echinostoma paraensei (Maldonado et al. 2001, 2003). Physa spp. are intermediate hosts for diplostomatid, echinostomatid, and strigeid trematodes and are laboratory hosts for nematodes in the genus Angiostrongylus (Malek 1980). In addition, Physa spp. serve as hosts to nematomorph worms and the oligochaete Chaetogaster sp., which are parasites of invertebrates (Gamble and Fried 1976, Hanelt et al. 2001). The public health or veterinary significance of populations of P. marmorata on Dominica are unknown, but further studies could prove this snail a host to helminths of economic significance. Noblet and Damian (1991) reported collecting Pliysa cubensis Pfeiffer, 1839 in Dominica. Pliysa cubensis is a junior synonym of Physa acuta Draparnaud, 1805 (Paraense and Pointier 2003). However, we collected P. marmorata in the same habitats and localities that Noblet and Damian (1991) reported P. acuta. These older reports of P. acuta thus probably represent misidentifications of P. marmorata.

The neritid snail Neritina punctulata Lamarck, 1816 was collected from both streams and fish ponds. Neritina punctulata occurs in streams throughout Dominica (Starmuhlner 1984) and breeds in streams with eggs attached to boulders. Neritina punctulata is possibly the largest freshwater snail on Dominica and is not known to harbor parasites of humans or domestic animals. However, this snail is eaten by humans on Dominica. As long as the snails are adequately cooked, they would pose no threat even if they were intermediate hosts to helminths or other infectious agents. A detailed study of the potential helminths or other infectious agents pathogenic to humans in N. punctulata will thus have public health implications.

Exotic snails continue to be introduced into Dominica. There is at least one tropical fish store on the island, and tropical ampullarids, physids, and planorbids are sold by the tropical fish industry. Freshwater plants such as water lettuce (*Pistia stratiotes*) are transported from one Caribbean Island to another by travelers and are used as ornamental plants in local water gardens or fishponds. Snails such as *Biomphalaria* spp. can be transported on these plants.

ACKNOWLEDGMENTS

We thank J. Andre for issuing collection and research permits, P. H. Adler, R. W. Blob, J. A. Korecki, B. A. Powell, P. D. McMillan, A. G. Wheeler, Jr., and N. G. L. Osler for logistical and collecting assistance in Dominica, and the American Society for Microbiology for partially supporting this research. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

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Accepted: 5 March 2007; **final corrections received:** 2 November 2007